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Brief Clinical Report

Chromosome Instability With Bleomycin and X-Ray Hypersensitivity in a Boy With Nijmegen Breakage Syndrome

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We report on a Mexican boy with microcephaly, short stature, and a high frequency of chromosome aberrations with rearrangements involving chromosomes 7 and 14, typical of ataxia telangiectasia (AT) patients. He had neither ataxia nor telangiectasia, and his immunological status and serum alpha feto protein (AFP) level were normal. Bleomycin hypersensitivity, which has been demon-strated in AT patients, was tested in the patient using AT and normal subjects forcomparison. The frequency of spontaneously occurring chromosome aberrations in lymphocyte cultures was significantly higher in the patient and the AT patient than in the normal subject. Four cells from the patient showed structural rearrangements involving chromosomes 7 or 14, with breakpoints typical for AT. When exposed to 5.0 μg bleomycin, the lymphocytes from the AT patient showed the highest sensitivity to this agent; our patient had an intermediate sensitivity. In both patients several rearrangements involving chromosomes 7 and 14 were scored, while none were observed in the normal subject. A colony survival assay (CSA) [Huo et al., 1994: Cancer Res 54:2544–2547], using a lymphoblastoid cell line (LCL) derived from our patient, showed a survival fraction (SF) of 7%, which is in the same range as in AT patients. The clinical picture, together with the cytogenetic and radiosensitivity results, suggests that our patient fits the variable spectrum of Nijmegen breakage syndrome. Am. J. Med. Genet. 70:24–27, 1997.

KEY WORDS: ataxia telangiectasia; chromosome instability; Nijmegen syndrome; bleomycin

INTRODUCTION

Weemaes et al. [1981] described a chromosome instability syndrome called Nijmegen breakage syndrome (NBS), characterized by microcephaly, short stature, skin abnormalities, cellular and humoral immunological defects, and mental retardation. Cytogenetic studies show characteristic chromosome 7 and 14 rearrangements; the lymphocytes and fibroblasts show X-ray hypersensitivity [Taylor et al., 1975] and radioresistant DNA synthesis [Painter and Young, 1980; de Wit et al., 1981] similar to that described in classical ataxia-telangiectasia (AT). A further delineation of NBS in five unrelated families [Taalman et al., 1989] indicated that the immunological disturbances can be variable and that intelligence may be normal. Barbi et al. [1991] reported a microcephalic and growth-retarded girl with chromosome instability of the AT type, and with X-ray hypersensitivity, but lacking severe infections and immunological defects.

Classical AT includes different complementation groups (A, C, D, and E) that are clinically similar. Other AT-related chromosome breakage syndromes such as NBS (V1 and V2) share the cytogenetic and radiosensitivity characteristics of AT [Jaspers et al., 1988; Curry et al., 1989], as well as the increased incidence of malignancy of probands and heterozygous [Seemanová, 1990].

Here we report on a microcephalic Mexican boy whose clinical picture and cytogenetic and radiosensitivity results suggest the diagnosis of NBS.

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CASE REPORT

The patient was the product of the first uncomplicated pregnancy of a 20-year-old mother and father. There was no known parental consanguinity. At gestational age 40 weeks, birth weight was 2,500 g. In his first months, he suffered from several episodes of diarrhea due to apparent lactose intolerance, which disappeared with appropriate treatment. No history of recurrent infections was recorded.

On clinical examination at age 3 years, his height was 88 cm and weight was 11,600 g (both <3rd centile). His head circumference was 42 cm (<3rd centile). He had sparse hair, sloping forehead, mongoloid slant of palpebral fissures, broad nasal bridge, micrognathia, and multiple pigmented nevi (Fig. 1). Mild psychomotor retardation (developmental coefficient of 77%), mainly in language and adaptive areas, was diagnosed.

Laboratory Studies

Laboratory studies showed normal white and red counts, and normal serum concentrations of IgA, IgM, IgG, C3, and C4. Serum alpha feto protein (AFP) was 3.8 ng/ml. EEG showed interhemispheric asymmetry because of left decreased voltage. Karyotype was 46,XY; however, a frequency of 0.3 spontaneous aberrations per cell was found; two aberrations were: inv(7)(p13;q34) and t(7;14)(q34;q11). The mother's karyotype was normal; the father was not available for study.

These results suggested the diagnosis of NBS, and the following tests were performed.

MATERIALS AND METHODS

Cytogenetics and Bleomycin Sensitivity

Lymphocytes from peripheral blood samples were obtained from the patient, from a known AT patient, and from a normal subject.

Cultures were prepared in flasks containing 0.4 ml of whole heparinized blood, 5 ml of supplemented McCoy's 5a medium, 0.25 ml of phytohemagglutinin, and antibiotics. Half the cultures of each subject were exposed to 5.0 μg/ml bleomycin during the last 24 hr. We selected this dose based on experiments, done in our laboratory, where bleomycin was used to induce chromosome aberrations in lymphocytes from AT patients. The cells were harvested at 72 hr. Mitotic cells were obtained according to conventional procedures and G-banded with 5% Giemsa. Slides were coded to ensure blind analysis. Chromosome aberrations (chromatid and chromosome breaks, and structural rearrangements) were scored in 50 cells from each type of culture. The number of chromosome rearrangements involving chromosome 7 and/or 14 on breakpoints, typical for AT and NBS, was also recorded.

Statistical analysis was done using the Student-Welch t-test.

Colony Survival Assay

To better assess the radiosensitivity of peripheral blood cells derived from our patient, we carried out the

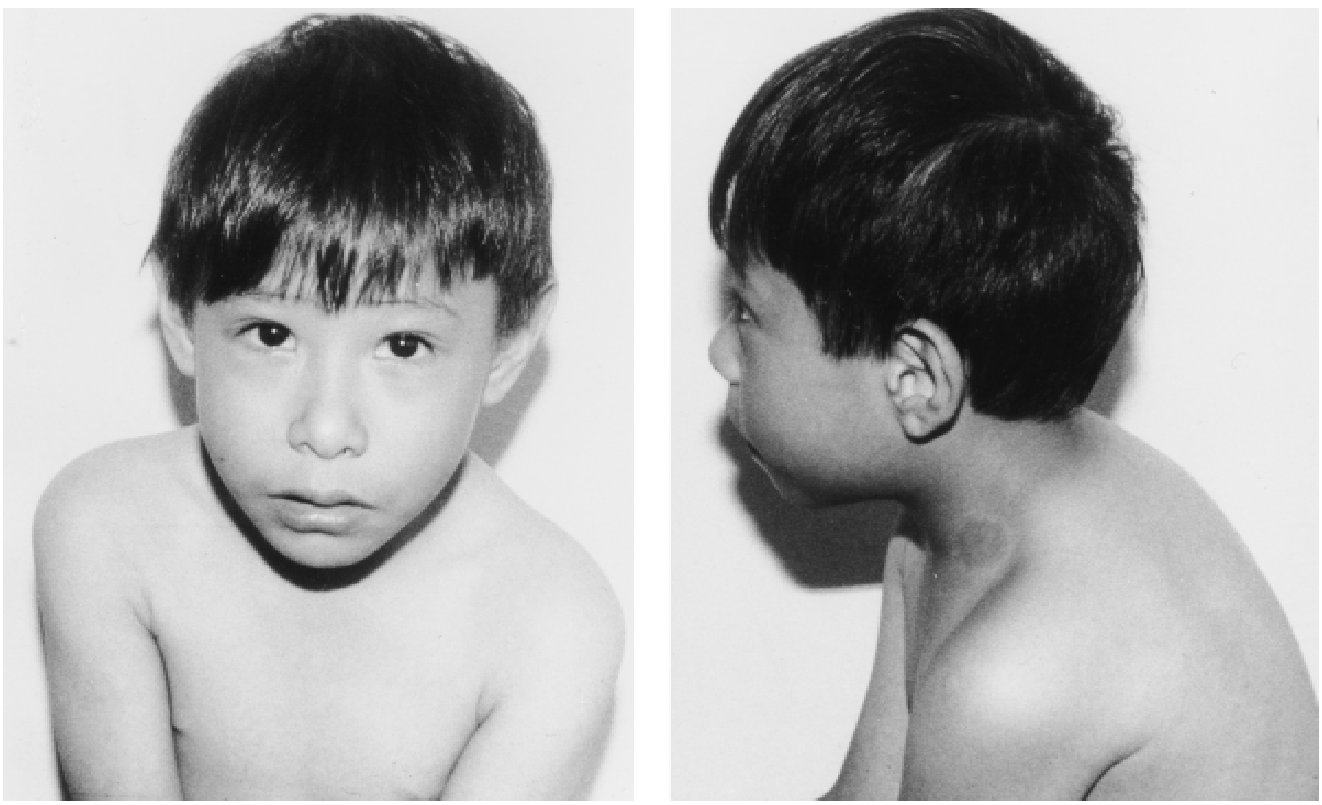

Fig. 1. Patient at age 3 years. Front and lateral views of face.

TABLE I. Spontaneous and Bleomycin-Induced Chromosomal Aberrations in the NBS Patient, an AT Patient, and a Normal Subject†

	Spontaneous		Bleomycin 0.5 μg	
Subject	$\bar{x}$ ab/cell (50)	No. of aberrations on chromosome 7 or 14‡	$\bar{x}$ ab/cell (50)	No. of aberrations on chromosome 7 or 14‡
NBS patient*	0.24	4	0.44	8
AT patient**	0.20	4	2.20	20
Normal***	0.02	0	0.08	0

†$\bar{x}$ ab/cell, average aberrations per cell.
‡Aberrations on chromosomes 7 or 14 involving breakpoints 7p13, 7q34, 14q11, or 14q32. In parentheses, number of cells analyzed.
*vs. ***, $P < 0.01$.
**vs. ***, $P < 0.01$.
*vs. ***, $P < 0.1$.
**vs. ***, $P < 0.001$.

colony survival assay (CSA), as described by Huo et al. [1994]. In brief, after Epstein-Barr transformation of lymphocytes, the immortalized lymphoblastoid cell line (LCL) derived from the patient was seeded into two flat-bottomed 96-well plates. One plate was irradiated at 1 Gy and the other was kept as a control. The plates were then incubated at 37°C in 5% CO_2 for 10–12 days. Viable cell colonies were identified by 3-[4,5dimethylthiazol-2-4]-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co., St. Louis, MO), adding 0.1 ml of a 1 mg/ml solution of MTT. After 2–4 hr of incubation, each well was analyzed under the microscope, looking for dark blue-stained viable cells. The presence of colonies with more than 32 cells was scored as a positive well. The survival fraction (SF) was calculated according to Huo et al. [1994].

RESULTS

The frequency of spontaneously-occurring chromosome aberrations in lymphocyte cultures of the patient and in the AT case was significantly higher than that observed in the normal subject ($P < 0.01$ and $P < 0.001$, respectively) (Table I). Four cells from the patient showed structural rearrangements involving chromosomes 7 or 14, where two cells were inv(7)(p13;q34), one was t(7;14)(q34;q11), and one was a break on 14q32. When exposed to 5.0 μg bleomycin, the lymphocytes from the 3 subjects showed an increased frequency of chromosome aberrations; the AT patient showed the highest sensitivity to this agent ($P < 0.001$ compared to normal), and our patient had an intermediate sensitivity ($P < 0.01$ compared to normal). In both patients, several rearrangements involving chromosomes 7 and 14 with characteristic breakpoints were scored (Table I). The more frequent chromosome rearrangements were inv(7)(p13;q34) and t(7;14)(q34;q11). To further support the NBS diagnosis, we repeated the test in the patient, using 7.5 μg of bleomycin. The results in 50 cells showed a frequency of 0.56 aberrations per cell, and 12 characteristic rearrangements of chromosomes 7 and 14.

CSA of LCL derived from normal subjects showed SF of 42.7 ± 11.7%, the same assay in AT LCLs showed 12.5 ± 4.8%, and our patient showed 7%, which is in the same range as AT patients [Huo et al., 1994].

DISCUSSION

The cytogenetic study of a microcephalic, mildly retarded boy with short stature showed a high frequency of chromosome aberrations, with rearrangements involving chromosomes 7 and 14 typical of AT and NBS patients. However, at age 3 years the patient had neither ataxia, telangiectasias, nor infectious problems; serum AFP was normal. The diagnosis of NBS was suggested on the basis of the cytogenetic findings in a patient with clinical findings which have been described in NBS patients [Taalman et al., 1989; Weemaes et al., 1994; Chrzanowska et al., 1995]; however, an immunological defect was not detected. In order to solve the diagnostic dilemma in our patient, we tested the lymphocyte culture for bleomycin sensitivity and the LCL for radiosensitivity, using CSA.

Hypersensitivity and resistance to DNA replication has been demonstrated for AT and NBS cells exposed to bleomycin [Taylor et al., 1979; Taalman et al., 1983; Jaspers et al., 1988; Li and Shiraishi, 1990]. The lymphocytes from our patient showed chromosome hypersensitivity to bleomycin, which was less pronounced than in the AT patient cells. In both patients, several typical AT and NBS aberrations involving chromosomes 7 and 14 were observed in the bleomycin-tested cultures; however, the proportion in relation to total aberrations was not higher than in control cultures.

Recently, Weemaes et al. [1994] reported that the percentage of chromosome 7 and 14 rearrangements was significantly higher in NBS patients than in AT patients. In this report, control lymphocyte cultures did not show this difference. When tested for hypersensitivity to bleomycin (0.5 μg), the frequency of total induced aberrations was higher in the AT patient than in the NBS patient; however, the proportion of chromosome 7 and 14 aberrations was higher in the NBS than in the AT patient (8/22 vs. 20/110 total aberrations).

The LCL derived from our patient was tested for radiosensitivity by CSA and showed a postradiation survival fraction in the same range as for AT and NBS patients [Huo et al., 1994]. CSA measures the general lethal effects of radiation and clearly distinguishes AT from normal cells. The postradiation survival fractions of LCLs derived from other AT-related syndromes such as NBS (V1 and V2) are in the same AT range as in our patient, suggesting that these X-ray-sensitive syn-

dromes may share a common mechanism, related to the repair of radiation damage to DNA [Huo et al., 1994].

A gene causing AT was identified on 11q22–23 [Savitsky et al., 1995], and this may aid in diagnosis. However, the genotyping results with polymorphic microsatellite DNA markers on the AT region in six families with AT-V1 and AT-V2 indicate that these AT variants are genetically distinct from classical AT [Stumm et al., 1995].

The clinical and laboratory findings in the present patient are very similar to those reported by Barbi et al. [1991] in a microcephalic and growth-retarded girl with no signs of telangiectasias, ataxia, or immunodeficiency, but showing AT-type chromosome instability, radiation hypersensitivity, and radioresistant DNA synthesis. Although we could not perform the radioresistant DNA-synthesis test, the data presented here support the contention that the patient may fit the variable spectrum of NBS. It would be interesting to assess the complementation group, but this test is not generally available.

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